Neutral deinking with a deinking composition comprising a lipase and a fatty acid ester

Cross-Reference To Related Applications

This application is a continuation of U.S. application no. 10/050,489, filed January 16, 2002, which claims the benefit of U.S. provisional application no. 60/261,784, filed on January 16, 2001, the contents of which are hereby incorporated by reference.

Technical Field

The present invention relates, in general, to deinking of wastepaper. In particular, the present invention relates to a deinking process at neutral (pH 4-8.5) conditions.

Background of the Invention

Wastepaper has long served as a source of raw fiber material for papermaking. It has been standard practice in the art to reclaim wastepaper to allow the reclaimed paper fibers to be used as part or all of the stock of subsequent production of a variety of paper and paperboard products. Today, greater utilization of reclaimed fibers has provided incentive for taking steps to upgrade the reclaimed products. These steps include treatment to effectively remove ink from waste fibers in order to permit their use in the manufacture of e.g. newsprint and high quality papers. Increasing amounts of e.g. old newspapers (ONP) and waste magazines (WM) are becoming available with increased participation of end consumers in recycling.

In the course of conventional paper reclamation, deinking procedures include steps for converting the wastepaper to pulp and contacting the pulp with an alkaline aqueous deinking medium containing a chemical deinking agent. The mechanical action and the alkalinity of the aqueous medium cause the partial removal of ink from the pulp fiber. The deinking agent completes this removal and produces an aqueous suspension and/or dispersion of the ink particles. The resulting mixture is subsequently treated to separate the suspended/dispersed ink from the pulp. This separation may be by flotation and/or washing techniques known in the art.

Conventional deinking chemicals comprise a complex mixture of chemicals, e.g. sodium hydroxide, sodium silicate, chelating agents, hydrogen peroxide, surfactants, dispersants, collector chemicals and agglomeration chemicals. In general, it is standard in the prior art methods to include a significant amount of alkaline material, since it is believed that the alkaline material is needed for sufficient saponification and hydrolysis of the ink resins. In addition, mention is made of the fiber swelling by the caustic being partially responsible for the detachment of ink particles from the fiber surface. Typically, the pH during such a deinking process is from about 9.5 to about 11. Exposing the cellulosic and lignocellulosic fibers to this degree of alkalinity tends to cause yellowing of the fibers and, therefore, it is generally necessary to add an oxidative or reductive bleaching agent, such as peroxide. Furthermore, the alkaline method causes irreversible changes to the pulp fibers, and hence represents a cost to the facility

Thus, there is a need for a deinking process that operates under slightly acidic (from about pH 4) to slightly alkaline (to about pH 8.5) or neutral conditions, which is safe and which is economically and environmentally desirable.

It has been found by the present inventors that the use of a deinking agent, which comprises a lipase and a fatty acid ester, fulfills the above-mentioned requirements to such a deinking process.

Summary of the Invention

Thus, the present invention relates to a method for deinking wastepaper comprising the steps of

i) pulping the wastepaper at a pH between 4 and 8.5 in the presence of deinking agent comprising a lipase and a fatty acid ester; and

ii) removing the thereby dislodged ink particles.

Detailed Description of the Invention

The deinking agent suitable for the purposes described herein should include a lipase, which is active in the pH interval of from about 4 to about 8.5.

The lipase enzyme to be used in the present invention is one that can hydrolyze ester bonds. Such enzymes include, for example, lipases, such as triacylglycerol lipase (EC 3.1.1.3), lipoprotein lipase (EC 3.1.1.34), monoglyceride lipase (EC 3.1.1.23), lysophospholipase, ferulic acid esterase and esterase (EC 3.1.1.1, EC 3.1.1.2). The numbers in parentheses are the systematic numbers assigned by the Enzyme Commission of the International Union of Biochemistry in accordance with the type of the enzymatic reactivity of the enzyme.

The lipase may be a microbial lipase, e.g. from bacteria or fungi such as *Humicola* or *Pseudomonas*, particularly lipase from *H. lanuginosa* (this lipase being referred to as Resinase A 2x®).

Preferred microbial lipases to be used in the methods of the present invention may be of bacterial, yeast or fungal origin, and suitable examples include a lipase derived or obtainable from a strain of *Humicola* spp., *Rhizomucor* spp., *Candida* spp., *Aspergillus* spp., *Rhizopus* spp. or *Pseudomonas* spp., especially from a strain of *H. lanuginosa*, *Rh. miehei*, *C. antarctica*, *Aspergillus niger* or *Pseudomonas cepacia*. Specific examples of such lipases are lipase A and lipase B of *C. antarctica*, described in WO 88/02775, the *Rh. meihei* lipase in EP 238 023, the *H. lanuginosa* lipase described in EP 305 216, and the *P. cepacia* lipase described in EP 214 761 and WO 89/01032.

The lipase may be a native enzyme found in nature, or it may be a variant thereof obtained by altering the amino acid sequence. Examples of such variants are those described in WO 92/05249, WO 94/25577, WO 95/22615, WO 97/04079 and WO 97/07202, WO 98/08939, PCT/DK99/00068, EP 99610010.3 and Danish patent application PA 1999 00441.

A specific example of a variant is the lipase from *Humicola lanuginosa* strain DSM 4109 having the mutations E1SPPCGRRP, E99N, N101S, E239C, Q249R.

Specific examples of suitable, commercially available, lipases are, e.g., Resinase A 2x, Novozyme 735, and Novozyme 525 (all available from Novozymes A/S, Denmark).

The lipase used for the present invention may be obtained from the microorganism in question by use of any suitable technique. For instance, a lipase preparation may be obtained by fermentation of a microorganism and subsequent isolation by a method known in the art, but more preferably by use of recombinant DNA techniques as known in the art. Such method normally comprises cultivation of a host cell transformed with a recombinant DNA vector capable of expressing and carrying a DNA sequence encoding the lipase in question, in a culture medium under conditions permitting the expression of the enzyme and recovering the enzyme from the culture. The DNA sequence encoding the lipase to be used may be of any origin, e.g. a cDNA sequence, a genomic sequence, a synthetic sequence or any combination thereof. Examples of suitable methods of preparing microbial lipases are described in, e.g. EP 0 238 023 and EP 0 305 216.

Lipase Units (LU) are determined according to the following assay: Lipase activity (LU) is assayed using glycerine tributyrat as a substrate and gum-arabic as an emulsifier. 1 LU (Lipase Unit) is the amount of enzyme which liberates 1 µmol titratable butyric acid per minute at 30°C, pH 7.0. The lipase activity can be assayed by pH-stat titration using e.g. a standard Radiometer titrator VIT90, obtainable from Radiometer, Copenhagen.

The lipase is typically added to the pulp in an amount corresponding to 0.001% - 0.15% % by weight of the dry pulp, preferably in an amount corresponding to 0.010 – 0.10 % by weight of the dry pulp, such as from 0.015 – 0.075% by weight of the dry pulp. These weight numbers may, of course, vary considerably depending of the enzyme used, the specific activity of the enzyme, the applied pH, temperature, etc. It is, however, in general preferred that the lipase is added in an amount corresponding to at least 500 LU/kg dry wastepaper, preferably in the range 500 – 500,000 LU/kg waste paper.

Further, the deinking agent suitable for the purposes described herein should include a fatty acid ester.

In one embodiment of the invention, the fatty acid ester suitable for the purposes described herein may be a methyl ester, an ethyl ester, a *n*-propyl ester, an isopropyl ester, a *n*-butyl ester, an isobutyl ester, a *sec*-butyl ester, a *tert*-butyl ester, a monoglyceride, a diglyceride or a triglyceride of a C_6-C_{22} fatty acid, the C_6-C_{22} fatty acid being optionally substituted with one or more hydroxy, ethoxy, *n*-propoxy and/or isopropoxy groups. In a preferred embodiment the fatty acid ester is a triglyceride.

In another embodiment of the invention, the fatty acid ester suitable for the purposes described herein is a C_6-C_{22} fatty acid, which has been alkoxylated with ethylene oxide, propylene oxide, or a combination thereof. In a preferred embodiment the fatty acid ester is a C_6-C_{22} fatty acid, which has been alkoxylated with ethylene oxide and propylene oxide of the general formula

$$HO\text{-}(CH_2\text{-}CH_2\text{-}O)_n\text{-}(CH2\text{-}CH(CH_3)\text{-}O)_m\text{-}H,$$

Wherein n is an integer in the range from 3 to 25, and m is an integer in the range from 2 to 14.

An example of commercially available fatty acid esters of the above structure would include the Nopalcol® series(available from Henkel Corp..)

When used herein the term "C_6-C_{22} fatty acid" is intended to mean a (not substituted) saturated fatty acid or an (not substituted) unsaturated fatty acids comprising one or more double bonds, having a total number of carbon atoms of from 6 to 22. Thus, the C_6-C_{22} fatty acid may, in addition to the terminal carboxylic acid group, contain a linear or branched alkyl, hydroxyalkyl, alkenyl or hydroxyalkenyl radical. As will be acknowledged by the skilled person, in case the fatty acid is substituted with, for example, ethoxy, *n*-propoxy and/or isopropoxy groups, the total number of carbon atoms in the fatty

acid moiety of the fatty acid ester may be larger, in some cases considerably larger, than 22.

Specific examples of fatty acid moieties include caproic acid (6:0), enanthic cid (7:0), caprylic acid (8:0) pelargonic acid (9:0), capric acid (10:0) undecylenic acid (11:0), lauric acid (12:0), tridecylic acid (13:0), myristic acid (14:0), palmitic acid (16:0), stearic acid (18:0), palmitoleic acid (16:1), oleic acid (18:1), elaidic acid (18:1), ricinoleic acid (18:1), linoleic acid (18:2), linolenic acid (18:3) and mixture thereof, wherein the first number in the brackets indicates the total number of carbon atoms in the fatty acid moiety, whereas the second number indicates the number of double bonds.

In an embodiment of the invention the fatty acid ester (or more precisely, the C_6-C_{22} fatty acid moiety of the fatty acid ester) is substituted with one or more ethoxy and/or isopropoxy groups. In a preferred embodiment, the substitutent has the general formulae:

$$-(O\text{-}CH_2\text{-}CH_2)_x\text{-}(O\text{-}CH(CH_3)\text{-}CH_2)_y\text{-}OH,$$
$$-(O\text{-}CH_2\text{-}CH_2)_x\text{-}(O\text{-}CH_2\text{-}CH(CH_3))_y\text{-}OH,$$
$$-(O\text{-}CH(CH_3)\text{-}CH_2)_y\text{-}(O\text{-}CH_2\text{-}CH_2)_x\text{-}OH, \text{ or}$$
$$-(O\text{-}CH_2\text{-}\ CH(CH_3))_y\text{-}(O\text{-}CH_2\text{-}CH_2)_x\text{-}OH,$$

wherein x is an integer in the range from 1 to 25, preferably in the range from 1 to 10, and y is an integer in the range from 1 to 10, preferably in the range from 1 to 5.

As will be understood by the skilled person, the fatty acid esters suitable for the purposes described herein may be any of the above fatty acid esters. Such esters may be used either individually or in admixture. For example, a number of commercially available fatty acid esters are available, which contains a number of the above-mentioned fatty acid esters. Specific examples of such commercially available esters (or fatty acids which can easily be converted to esters by standard method well known to the skilled person) include esters of tallow fatty acid, esters of butter fatty acids, esters of

coconut fatty acids, esters of cottonseed fatty acids, esters of lard fatty acids, esters of olive fatty acids, esters of palm fatty acids, esters of palm kernel fatty acids, esters of peanut fatty acid, esters of peanut fatty acids, esters of soybean fatty acids, and esters of castor oil, including ethoxylated and propoxylated derivatives thereof. A specific example of an ethoxylated and propoxylated derivative of castor oil triglyceride is the Hartaflot G-5000 (available from Huntsman Corp., Houston, TX, U.S.A.). The approximate distribution of various fatty acids the above-mentioned commercially available products can be found *inter alia* in Morrison and Boyd: Organic Chemistry. Fifth Edition. 1987, Allyn and Bacon, Inc., Boston, on page 1265.

The fatty acid ester is typically added to the pulp in an amount corresponding to 0.025 - 1% by weight of the dry pulp, preferably in an amount corresponding to 0.05 –0.75% % by weight of the dry pulp.

The fatty acid esters used for the purposes described herein are chemically unique compared to other surfactants commonly used in deinking wastepaper. Standard collector chemistry involves the use of a fatty acid. Such fatty acids (typically used in the form of the calcium salt) collect the ink upon liberation from the fibers, and keep them from being reattached to the fibers. Such fatty acid salts also allow the liberated ink to be removed in the froth during the flotation stage, while the fibers remain behind.

The fatty acid esters described herein constitute a substrate for the lipase, i.e. the fatty acid ester will, during pulping, be gradually converted into the corresponding fatty acid and the corresponding alcohol. This means that the added fatty acid/fatty acid ester system functions as a "displector" class of surfactant. It serves both to disperse the ink from the fiber surface as well as to collect the ink to allow its removal during flotation. As it appears from the examples herein, blank experiments have revealed that the lipase alone, i.e. without addition of fatty acid ester, did not dislodge ink particles from the fiber surface.

Clearly, the double function of the fatty acid ester confers the advantage that further dispersant and/or collectors may not be necessary.

The deinking agent would usually be supplied to the waste paper initially, i.e. when the pulping stage commences. Alternatively, the deinking agent may be supplied to waste-paper which is already in the form of a pulp, that is, to wastepaper which has first been substantially reduced to individual fibers. In the former case, the pulping step where the deinking agent is present is also sometimes termed "re-pulping".

The lipase and the fatty acid ester of the deinking agent are prepared by conventional means. The lipase and the fatty acid ester may be added to the waste paper either when pulping is initiated or may be supplied to the wastepaper which is already in the form of a pulp, that is, to wastepaper which has first been substantially reduced to individual fibers. The lipase and the fatty acid ester may be combined prior to addition or the components can be added to the wastepaper individually in any order.

Pulping can be conducted using any of the various conventional processes and equipment designed for this purpose. Most conveniently, the wastepaper process feedstock is treated in a device known as a "hydrapulper", which produces a slurry of the fibers in water.

Independently of whether the deinking agent is added to the wastepaper when the pulping stage commences or is added in a re-pulping stage, it is preferred that the pulping with the deinking agent is carried out for at the most 60 minutes, in particular for at the most 50 minutes, e.g. for at the most 40 minutes, such as for at the most 30 minutes, more preferably for at the most 20 minutes, such as for at the most 15 minutes, e.g. for about 10 minutes.

It is important to maintain an appropriate pulp slurry temperature while the deinking agent is contacted with the pulp slurry. The temperature has to be consistent with the activity temperature profile of the lipase, and preferably the process is run at a tempera-

ture where the employed lipase has maximum activity. Since a number of commercially available lipases have a substantial activity in the temperature range of from 25 to 75°C it is contemplated that the process can be run using temperatures, which do not deviate substantially from the temperatures normally used in such processes. Typically, pulping with the deinking agent is carried out at a temperature from 25 to 75°C, preferably from 30 to 70°C, such as from 35 to 65°C, e.g. from 40 to 60°C, more preferably from 45 to 60°C, such as from 45 to 55°C, e.g. about 50°C.

The efficiency of the deinking agent can be significantly influenced by the pH of the pulp slurry while contacting the deinking agent with the pulp slurry, since fluctuations in the pH can result in deactivation of the lipase. As indicated above, the pH of the pulp slurry should be in the range of from 4 to 8.5. In a preferred embodiment of the invention, the pulping with the deinking agent is carried out at a pH between 4.5 and 8.5, in particular between 5 and 8.5, such as between 5.5 and 8.5, more preferably between 6 and 8.5, such as from 6.5 to 8.5, e.g., between 7.5 and 8.5. The pulp slurry pH has to be consistent with the activity pH range of the lipase, and in a preferred embodiment the process is run at a pH where the employed lipase has optimal activity. The pH of the pulp may be adjusted by means of buffering agents, such as sodium citrate, sodium carbonate, sodium phosphate and the like. It is particularly preferred, however, that hydroxides, in particular alkali metal hydroxides, such as sodium hydroxide, is not added at any stage, i.e. prior to, during or after pulping.

The waste paper to be deinked according to the invention may be any reclaimed fiber, such as old newspapers (ONP), waste magazines (WM), mixed and sorted office waste, computer print outs, white ledger waste paper, etc.

In a preferred embodiment of the invention the wastepaper comprises ONP, WM or a combination thereof.

In one embodiment of the invention the amount of ONP constitutes at least 10% by weight of the total amount of wastepaper, preferably at least 20% by weight, e.g. at

least 30% by weight, e.g. at least 40% by weight, more preferably at least 50% by weight, such as at least 60% by weight, e.g. at least 70% by weight, most preferably at least 80% by weight, such as at least 90% by weight, e.g. at least 95% by weight of the total amount of wastepaper. In a further embodiment of the invention the wastepaper consists essentially of ONP.

In another embodiment of the invention the amount of WM constitutes at least 10% by weight of the total amount of wastepaper, preferably at least 20% by weight, e.g. at least 30% by weight, e.g. at least 40% by weight, more preferably at least 50% by weight, such as at least 60% by weight, e.g. at least 70% by weight, most preferably at least 80% by weight, such as at least 90% by weight, e.g. at least 95% by weight of the total amount of wastepaper. In a further embodiment of the invention the wastepaper consists essentially of WM.

In a still further embodiment of the invention the wastepaper comprises 1-60% by weight of WM and 40-99% by weight of ONP, preferably 10-50% by weight of WM and 50-90% by weight of ONP, such as 20-50% by weight of WM and 50-80% by weight of ONP, e.g. 30-50% by weight of WM and 50-70% by weight of ONP.

The consistency of the pulp will typically be in the range from about 0.5% to about 15%, preferably from 1 to 15%, such a from 2 to 15%, e.g. from 4 to 15%, more preferably from 6 to 15%, such as from 8 to 15%, e.g. from 8 to 14%.

In addition to water, pulp and deinking agent, the pulp may further contain substances conventionally employed in deinking process, e.g. brightening agents, solvents, anti-foam agents and water softeners, in particular brightening agents.

Although not particularly preferred the pulp may also contain additional surfactants, such as non-ionic and cationic surfactants. Examples of non-ionic surfactants are, e.g., alkoxylated fatty acids, such as DI 600® from High Point Chemical Corp.; alkyl phenyl ethers of polyethylene glycol, such as the Tergitol® series from Union Carbide; alkyl-

phenolethylene oxide condensation products, such as the Igepal® series from Rhone Poulenc; and aryl alkyl polyether alcohols, such as Rohm and Haas' Triton® X 400 series, e.g. Trition® X 100. Examples of cationic surfactants include imidazole compounds, such as Amasoft® 16-7 and Sapamine® P from Ciba-Geigy and quaternary ammonium compounds, such as Quaker® 2001 from Quaker Chemicals and Cyanatex® from American Cynamid.

The overall deinking process generally comprises pulping or maceration of the waste-paper and ink removal by a flotation system, a water washing system or a combined flotation/washing system, A screening or coarse cleaning stage can be utilized to remove contaminants such as glass, stone, metal and staples. A centrifugal cleaning stage (or stages) can be utilized to remove light weight materials such as plastic. Typical deinking processes are described in L.D. Fergusen "Deinking Chemistry: part 1" July 1992 TAPPI Journal pp. 75-83; L.D. Fergusen "Deinking Chemistry: part 2" August 1992 TAPPI Journal pp. 49-58; and J.L. Spielbauer "Deinking System Overview" Voith Inc. Appleton, pp. 1-9.

It is known from WO 95/14807 that, in the case of starch coated paper, the deinking effect can be improved by including a treatment with a starch-degrading enzyme and, consequently, in a further embodiment of the invention, the pulping is carried out in the presence of a starch degrading enzyme.

The starch-degrading enzyme is preferably an amylase, e.g. an α-amylase, a glucoamylase or a debranching enzyme. A single enzyme or a combination may be used, e.g. α-amylase together with glucoamylase and/or a debranching enzyme. Examples of preferred α-amylases are those derived from strains of *Bacillus*, e.g. *B. amyloliquefaciens* (*B. subtilis*), *B. licheniformis* or *B. stearothermophilus* and from strains of *Aspergillus*, e.g. *A. oryzae*. Examples of commercial products are BAN™, Termamyl®, Aquazyme Ultra™ and Fungamyl™ (products of Novozymes A/S).

Preferred glucoamylases are the glucoamylases derived from a strain of *Aspergillus niger,* e.g. the commercial product AMG (product of Novozymes A/S).

The debranching enzyme is preferably a pullulanase, particularly one derived from a strain of *Bacillus acidopullulyticus*, e.g. the commercial product Promozyme® (product of Novozymes A/S).

In addition, it is well known that cellulases may aid the deinking process. Furthermore, it is well known that cellulases may improve the drainability of the paper pulp. Consequently, in a still further embodiment of the invention the pulping is carried out in the presence of a cellulase.

Such cellulases are typically be derived from bacteria and fungi, such as *Aspergillus niger, Trichoderma virde, Thielatia terrestris*, *Humicola sp.* and *Bacillus sp.* The cellulase may be a mono component or multi component cellulase, although mono component cellulases are preferred. A class of cellulases that are especially useful are cellulases lacking a cellulose binding-domain (CBD). Cellulose-binding domains have been described by P. Tomme et al. in J.N. Saddler & M.H. Penner (eds.), "Enzymatic Degradation of Insoluble Carbohydrates" (ACS Symposium Series, No. 618), 1996. A number of cellulases are known to contain a catalytic domain without a CBD; such a cellulase may be used as such in the invention. It is also known that other cellulases contain a catalytic domain and a CBD; such a cellulase may be truncated to obtain a catalytic core domain without the CBD, and this core may be used in the invention.

Cellulases may be classified into families on the basis of amino-acid sequence similarities according to the classification system described in Henrissat, B. et al.: *Biochem. J.*, (1991), **280**, p. 309-16, and Henrissat, B. et al.: *Biochem. J.*, (1993), **293**, p. 781-788. Some preferred cellulases are those belonging to Family 5, 7, 12 and 45.

A preferred Family 5 cellulase without CBD is an alkaline cellulase derived from a strain of *Bacillus*. One such Family 5 cellulase is the endo-glucanase from *Bacillus* strain KSM-

64 (FERM BP-2886). The cellulase and its amino acid sequence are described in JP-A 4-190793 (Kao) and Sumitomo et al., *Biosci. Biotech. Biochem.*, 56 (6), 872-877 (1992). Another Family 5 cellulase from *Bacillus* is the endo-glucanase from strain KSM-635 (FERM BP-1485). The cellulase and its amino acid sequence are described in JP-A 1-281090 (Kao), US 4,945,053 and Y. Ozaki et al., *Journal of General Microbiology*, 1990, vol. 136, page 1973-1979. A third Family 5 cellulase from *Bacillus* is the endo-glucanase from strain 1139. The cellulase and its amino acid sequence are described in Fukumori F. et al., *J. Gen. Microbiol.,* 132:2329-2335 (1986) and JP-A 62-232386 (Riken). Yet another preferred Family 5 cellulase without CBD is an endo-beta-1,4-glucanase derived from a strain of *Aspergillus*, preferably *A. aculeatus*, most preferably the strain CBS 101.43, described in WO 93/20193 (Novo Nordisk).

The Family 7 cellulase may be derived from a strain of *Humicola*, preferably *H. insolens*. An example is endo-glucanase EG I derived from *H. insolens* strain DSM 1800, described in WO 91/17244 (Novo Nordisk). The mature cellulase has a sequence of the 415 amino acids shown at positions 21-435 in Fig. 14 of said document and has a specific activity of 200 ECU/mg (based on pure enzyme protein). This cellulase may further be truncated at the C-terminal by up to 18 amino acids to contain at least 397 amino acids. As examples, the cellulase may be truncated to 402, 406, 408 or 412 amino acids. Another example is a variant thereof denoted endo-glucanase EG I* described in WO 95/24471 (Novo Nordisk) and having a sequence of 402 amino acids shown in Fig. 3 therein.

Alternatively, the Family 7 cellulase may be derived from a strain of *Myceliophthora*, preferably *M. thermophila*, most preferably the strain CBS 117.65. An example is an endo-glucanase described in WO 95/24471 (Novo Nordisk) comprising the amino acids 21-420 and optionally also the amino acids 1-20 and/or 421-456 of the sequence shown in Fig. 6 therein. As another alternative, the Family 7 cellulase may be derived from a strain of *Fusarium*, preferably *F. oxysporum*. An example is an endo-glucanase derived from *F. oxysporum* described in WO 91/17244 (Novo Nordisk) and Sheppard, P.O. et al., *Gene.* 150:163-167, 1994. The correct amino acid sequence is given in the latter reference. This cellulase has a specific activity of 350 ECU/mg.

A preferred Family 12 cellulase without CBD is CMC 1 derived from *Humicola insolens* DSM 1800, described in WO 93/11249 (Novo Nordisk). Another preferred Family 12 cellulase without CBD is EG III cellulase from *Trichoderma*, particularly *Trichoderma viride* or *Trichoderma reesei*, described in WO 92/06184 (Genencor). Alternatively, the Family 12 cellulase may be derived from a strain of *Myceliophthora*, preferably *M. thermophila*, most preferably the strain CBS 117.65. Such a cellulase (termed C173) can be produced by cloning DNA from CBS 117.65, and subsequently transforming *Aspergillus oryzae*, a non-cellulolytic host organism, and expressing the cellulase by cultivation of the transformed host, and separating the only cellulolytic active ingredient from the culture broth. C173 has optimum activity at pH 4-6.5, a specific activity of 226 ECU per mg protein and a molecular weight of 26 kDa (for the mature protein). The sequence of cDNA encoding C173 (from start codon to stop codon) and the amino acid sequence of the mature protein of C173 are shown in the sequence listing as SEQ ID NO: 1 and 2.

A preferred Family 45 cellulase without CBD is the EG V-core derived from *Humicola insolens*, described in Boisset, C., Borsali, R., Schulein, M., and Henrissat, B., FEBS Letters. 376:49-52, 1995. It has the amino acid sequence shown in positions 1-213 of SEQ ID NO: 1 of WO 91/17243 (Novo Nordisk). Another preferred Family 45 cellulase without CBD is FI-CMCase from *Aspergillus aculeatus* described by Ooi et al., Nucleic Acids Research, Vol. 18, No. 19, p. 5884 (1990).

Examples of commercially available cellulases include Novozym 613, Novozym 476, and Novozym 342 (all available from Novozymes A/S, Denmark)

The invention is further illustrated by the following non-limiting examples.

Materials and Methods

Equipment

Methrohm transportable pH meter 704; Hobart N-50 mixer equipped with a jacketed bowl attached to a constant temperature bath; Lamort 17 l laboratory flotation deinking device; Tappi handsteet mold and Tappi handshteet press; Macbeth Color-Eye 7000 spectrophotometer

Enzymes

Novozyme 735, Batch LDN00010., activity 5 KLU/g
Lecitase 10L, Batch L846-002, activity 10 KLU/g
Novozyme 525 L, Batch PPW 6354. activity 15 KLU/g
Resinase A 2x, activity 100 KLU/g

Chemicals and Raw Materials

The ONP for these experiments was prepared by tearing approximately 1" squares from a batch of Wall Street Journals that were less than two months old.
Hartaflot G-5000, an ethyloxylated and propoxylated castor oil derivative, was obtained from Huntsman Chemicals

Examples

Example 1 – Enzymatic Deinking

Hot tap water (750 ml) was placed in a jacketed Hobart mixer bowl with an external water bath set to provide a temperature of 50°C for the contents of the bowl. The Hartflot G-5000 (0.22 g) was added to the bowl. The wastepaper (165 g) was then added and allowed to wet out before agitation was initiated. After agitation was initiated an additional 750 ml of hot tap water was added stepwise in order to avoid splashing. Agitation was stopped and the pH of the pulp slurry was adjusted to approximately 6.5 by addition of 10% sodium carbonate.

Low speed agitation was then started and the lipase was added. Agitation was continued for 20 minutes after which the pulp was diluted 5 liter. The pulp was then added to the Lamort deinking cell (capacity: 17 liter). The cell was filled almost to the top, agita-

tion was initiated and less than 1 liter of pulp was removed to serve as the "pre-float" sample. The cell was then again filled to the top with hot tap water, the agitator was set to 1050-1100 rpm and the deinking process begun; During a 10 min. period the foam was scraped from the cell and collected to allow determination of the amount of rejects.

After the 10 min. flotation period the pulp was collected 1.2 g (approximately) hand-sheets were prepared from the pulp. The handsheets were prepared according to Tappi Test Method T-205. An additional set of handsheets was made, wherein the initially formed handsheets were backwashed by refilling the sheetmold with water and the handsheets were formed again. These handsheets are identified by the term "back-washed".

Brightness measurements of the air-dried handsheets were done using the Macbeth Coloreye unit equipped with the software to convert the optical data to Tappi Brightness values. The brightness readings were made on the smooth (wire) side of the hand-sheets. The individual handsheet being measured was backed up by using the stack other handsheets of the series while the readings were being made.

The observed results are compiled in the below Table 1.

Table 1

Run	Lipase/Surfactant	Observed pH		% Rejects	% Tappi Brightness		
		Initial	End		Pre-float	Post-float	Back-wash
1	None/None	6.5	6.2	4.5	43.3	46.1	49.0
2	87 mg Novozym 735/None	6.4	6.1	4.5	44.6	46.4	48.4
3	94.7 mg Novozym 735/0.23 g Hartaflot G-5000	6.3	5.8	6.3	44.0	48.0	50.2
4	55 mg Lecitase*/0.22 g Hartaflot G-5000	6.5	6.2	5.4	44.8	47.9	50.5
5	35 mg Novozym 525/0.22 g Hartaflot G-5000	6.6	6.3	7.8	43.6	47.1	49.0

* Lecitase is a phospolipase produced by Novozymes.

As it appears the lipases, when used alone, were practically unable to dislodge ink from the wastepaper. Furthermore, it can be seen that the lipase/fatty acid ester deinking agent is capable of dislodging significantly more ink (higher brightness values) as compared to the blank (no lipase and no surfactant) or the experiment, wherein only the lipase was added.

Example 2 – Surfactant Hydrolysis

Hartaflot G-5000 (0.42 g) was dissolved in 1500 ml water placed in a jacketed Hobart mixer bowl. The external water bath was set to provide internal temperatures close to those used for the earlier deinking trials. The initial pH was adjusted with 10% sodium carbonate to the value mentioned as the zero-time value in the table. Once adjusted, it was followed by the addition of lipase. 96 mg Novozym 735 was used and 24 mg Resinase A 2x were used, which correspond to the amounts used in the deinking trials. The pH change was monitored using a handheld pH meter and data was collected after 0, 10, 20, 40 and 60 min.

The observed results are compiled in the below Table 2.

Time (min)	0	10	20	40	60
Rate of hydrolysis by change of pH:					
Novozym 735	7.25	6.72	6.67	6.35	6.22
Resinase A 2x	7.79	7.68	7.52	7.35	7.25